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Antinociceptive Effect of Intrathecal Nefopam and Interaction with Morphine in Formalin-Induced Pain of Rats

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Background:

Nefopam, a non-opiate analgesic, has been regarded as a substance that reduces the requirement for morphine, but conflicting results have also been reported. The inhibition of monoamine reuptake is a mechanism of action for the analgesia of nefopam. The spinal cord is an important site for the action of monoamines however, the antinociceptive effect of intrathecal nefopam was not clear. This study was performed to examine the antinociceptive effect of intrathecal (i.t.) nefopam and the pattern of pharmacologic interaction with i.t. morphine in the formalin test.

Methods:

Male Sprague-Dawley rats were implanted with an i.t. catheter, and were randomly treated with a vehicle, nefopam, or morphine. Formalin was injected into the hind-paw 10 min. after an i.t. injection of the above experiment drugs. After obtaining antinociceptive ED_{50} of nefopam and morphine, the mixture of nefopam and morphine was tested for the antinociceptive effect in the formalin test at a dose of 1/8, 1/4, 1/2 of ED_{50}, or ED_{50} of each drug followed by an isobolographic analysis.

Results:

Intrathecal nefopam significantly reduced the flinching responses in both phases of the formalin test in a dose-dependent manner. Its effect, however, peaked at a dose of 30 μg in phase 1 (39.8% of control) and 10 μg during phase 2 (37.6% of control). The isobolograhic analysis indicated an additive interaction of nefopam and morphine during phase 2, and a synergy effect in antinociception during phase 1.

Conclusions:

This study demonstrated that i.t. nefopam produces an antinociceptive effect in formalin induced pain behavior during both phases of the formalin test, while interacting differently with i.t. morphine, synergistically during phase 1, and additively during phase 2. (Korean J Pain 2013; 26: 14-20)

Key Words:

antinociception, formalin, interaction, morphine, nefopam.

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INTRODUCTION

Nefopam has been known to be a non-opiate analgesic, and is structurally unrelated to other analgesics like NSAIDs [1]. Although supportive data is still lacking, the inhibition of monoamine reuptake has been suggested as a mechanism for the antinociceptive effect of nefopam [2,3]. It has been widely used, mainly in Europe, for controlling acute postoperative pain and other chronic pain conditions due to its relatively favorable side effects, while also providing beneficial properties like the morphine sparing effect [3-5]. However, different results have been reported on the interactions of morphine and nefopam [8,6-12]. In addition, most of the studies on its antinociceptive effect and the interactions with other analgesics were performed with the systemic administration of nefopam. Spinal nefopam was also shown to have an antinociceptive effect [13,14]. The spinal cord is also an important site of action for monoamines, which mediates descending pain modulation, the direction of which could be either inhibitory or facilitatory, depending on the pain stimuli [15]. In view of the mechanism of nefopam involving the inhibition of monoamine uptake, the nature of the pharmacologic interaction with morphine at the spinal level could be different according to the pain stimuli applied. This study examined the interaction of the intrathecal administration of morphine and nefopam using an isobolographic analysis in the formalin test in which two distinct phases of pain behavior are derived from different mechanisms.

MATERIALS AND METHODS

1. Animals and intrathecal catheter implantation

Male Sprague-Dawley rats weighing 225-250 g were used, with all animals being housed in a room with a constant temperature of 22-23°C and an alternating 12 h light/dark cycle. Water and food were allowed with no limitations. All experiments were performed according to the IASP guidelines for the Use of Animals in Research. The protocol was approved by the Institutional Animal Care and Use Committee, Research Institute of Medical Science, Chonnam National University Medical School.

Animals were implanted with a polyethylene-5 (PE-5) catheter into the i.t. space for the administration of the experiment drug according to the previous study [16]. Under general anesthesia using sevoflurane, a PE-5 catheter was introduced through the atlanto-occipital membrane and was advanced caudally, 8.5 cm, to the level of the lumbar enlargement. The other end was externalized through the skin of the top of the head and plugged with a stainless steel wire for drug administration. Rats with a neurological deficit after the catheter implantation were sacrificed immediately with an overdose of inhalational anesthetics. Rats were housed in individual cages after surgery.

2. Nociceptive test and behavioral study

Animals were injected subcutaneously with 50 µl of 5% formalin into the center of the hind-paw of the rat using a 30 gauge needle. The formalin test was conducted with the rats restrained in a cylinder. Intraplantar formalin injection produced a typical flinching response, which has two distinct phases. An initial acute phase (phase 1) was a relatively short quiescent period, followed by a prolonged tonic response (phase 2 beginning about 10 min after the formalin injection). Phase 1 represents acute nociception, while phase 2 is thought to involve the central sensitization of the dorsal horn neurons, as well as the sensitization of the peripheral neurons [17]. Flinching responses were quantified by counting the number of responses at 1 and 5 min (phase 1, 0-9 min), and every 5 min up to 60 min thereafter, after the injection of formalin (phase 2, 10-60 min). Each count was done for 1 min. The person who carried out the behavioral testing was blind to the treatment.

3. Antinociceptive effect of intrathecal nefopam and morphine

The antinociceptive effect of spinal nefopam hydrochloride (Pharmbio, Korea) and morphine sulfate (Sigma Aldrich, USA) were tested in the formalin test. Animals were placed in a restraint cylinder and allowed to adapt for 20 min before being allocated to receive one of the experimental drugs. They were randomly given nefopam (1, 3, 10, 30 µg), morphine (1, 3, 10, 30 µg), or saline through the i.t. catheter 10 min prior to the formalin injection into the hindpaw. Drugs were injected using a hand gear-driven Hamilton syringe with a volume of 20 µl followed by a flushing with a 10 µl vehicle.

4. Isobolographic analysis of interaction of nefopam with morphine

On completion of the antinociceptive study, dose-responsiveness was analyzed and ED_{50} (a dose that produced

a 50% reduction in the number of flinches compared with the control group) values for each drug were calculated. To calculate the ED_{50} of each drug, the number of flinches was converted to a percentage control as follows: % of control = [(sum of phase 1 or 2 flinch count with drug) / (sum of control phase 1 or 2 flinch count)] × 100. ED_{50} of each drug and its confidence interval were calculated using a standard linear regression analysis of a dose-response curve, according to the method by Tallarida [18]. Then, an isobolographic analysis was performed during both phases in order to determine the pattern of the pharmacologic interactions between nefopam and morphine. In brief, after obtaining the ED_{50} values of each drug, the mixture of nefopam and morphine were intrathecally co-administered at a dose of the ED_{50} values and fractions (1/2, 1/4, 1/8) of ED_{50} for each drug. The mixture was delivered intrathecally 10 min before the formalin test. The ED_{50} values of the mixture (experimental ED_{50}) were calculated from the dose-response curves of the mixtures. The ED_{50} values of each single agent were plotted on the X and Y axes respectively to construct the isobologram. Then, the theoretical additive dose combination of morphine and nefopam (theoretical ED_{50}) was calculated.

5. Statistical analysis

All data are expressed as mean ± SEM. The time-response data of the antinociceptive study are presented as the number of flinches. The dose-response data are expressed as the sum of flinches for each phase. Data from phase 1 and phase 2 were analyzed separately and compared with the control using a one-way analysis of variance followed by Bonferroni test for multiple comparisons. This was done to evaluate the dose-response relationship. The difference between the theoretical ED_{50} and the experimental ED_{50} was analyzed using the method reported by Tallarida. A P value < 0.05 was considered to be statistically significant.

RESULTS

1. Effects of intrathecal nefopam and morphine on formalin-induced pain

Animals treated with an i.t. vehicle exhibited the typical biphasic flinching responses that occur after the injection of formalin into the hindpaw. Intrathecal administration of morphine 10 min prior to the injection of for-

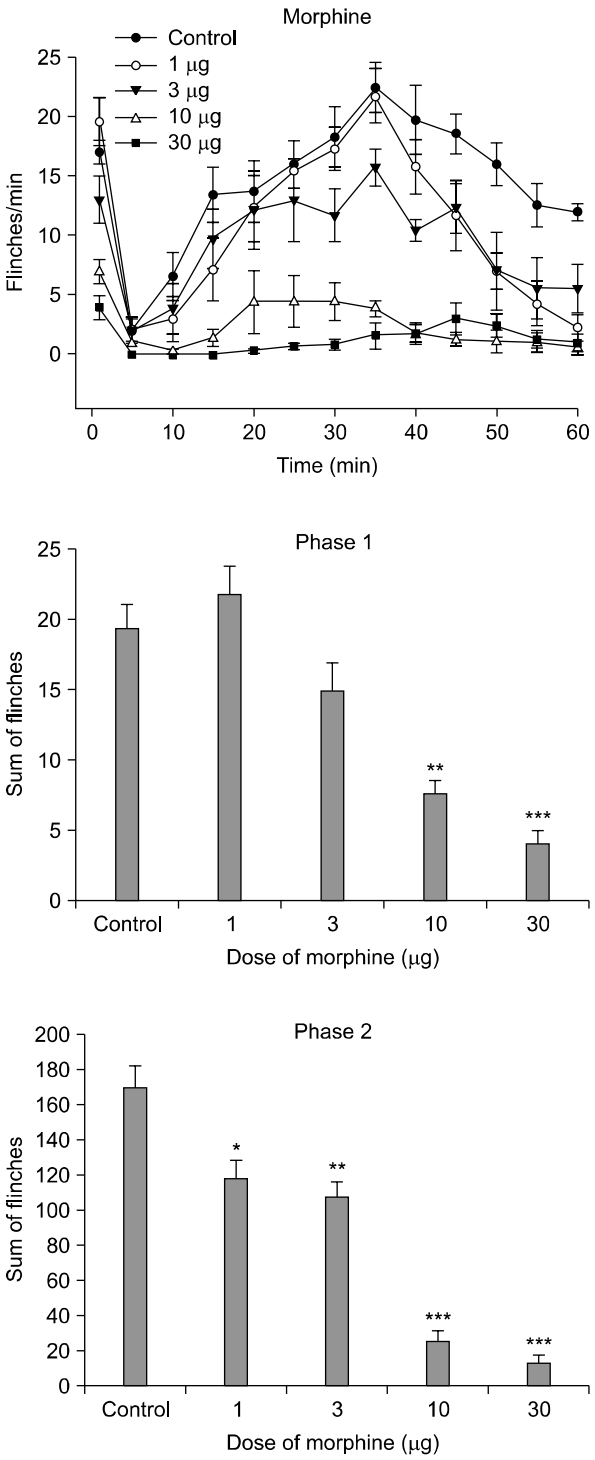

Fig. 1. Formalin induced flinching responses are attenuated dose-dependently by intrathecal injection of morphine in a dose-dependent manner. Time course after formalin injection (top panel), and dose response curve (middle and bottom) are shown. Each line represents the mean ± SEM of 6-8 rats/ group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control.

malin significantly reduced the flinching responses at both phases in a dose-dependent manner (Fig. 1). Intrathecal nefopam also significantly attenuated the flinching responses in both phases of the formalin test when compared to the control group (Fig. 2). The antinociceptive effect of intrathecal nefopam was dose-dependent, but the maximum effect was achieved at a dose of 30 μg in phase 1 (39.8% of control), 10 μg in phase 2 (37.6% of control), while higher doses produced no difference in the antinociceptive effect. This finding is consistent with the ceiling effect, which was shown in previous studies.

The ED_{50} value (95% confidence intervals) of i.t. nefopam was 13.7 (7.9–23.5) μg during phase 1, which is about 1.7-fold larger than the ED_{50} of i.t. morphine, 8.0 (5.5–11.5) μg. During phase 2, the ED_{50} of i.t. nefopam was 10.5 (5.0–21.8) μg, which is about 2.5-fold larger than the ED_{50} of i.t. morphine, 4.0 (3.0–5.2) μg.

2. Antinociceptive interaction of nefopam with morphine

The isobolographic analysis showed that the experimental ED_{50} (4.5 (3.5–5.8) μg) was significantly lower than the calculated theoretical ED_{50} (10.8 (7.0–14.6) μg) during phase 1, which indicated a synergistic interaction between nefopam and morphine (Fig. 3). In contrast to phase 1, co-administration of morphine and nefopam produced an additive antinociceptive interaction by showing no significant differences between the theoretical (7.2 (3.5–11.0) μg) and the experimental ED_{50} (4.6 (3.4–6.1) μg) of phase 2.

DISCUSSION

Multimodal analgesia is widely accepted as a good strategy to obtain optimal levels of analgesia, while reducing opioid-induced side effects [19]. The underlying mechanism of the multimodal analgesia is that the non-opioid drug has a different mode of analgesic action from the opioid, thus allowing the dose of opioid to be reduced, which results in the lowering of side effect incidences. The basic requirement for the employment of this strategy is the synergistic, or at least, an additive interaction between an opioid and another non-opioid drug.

The analgesic effects of systemic nefopam have been demonstrated in animal and clinical studies. However, the mechanism of action remains to be elucidated. Inhibition of monoamine reuptake, including serotonin and catechol-

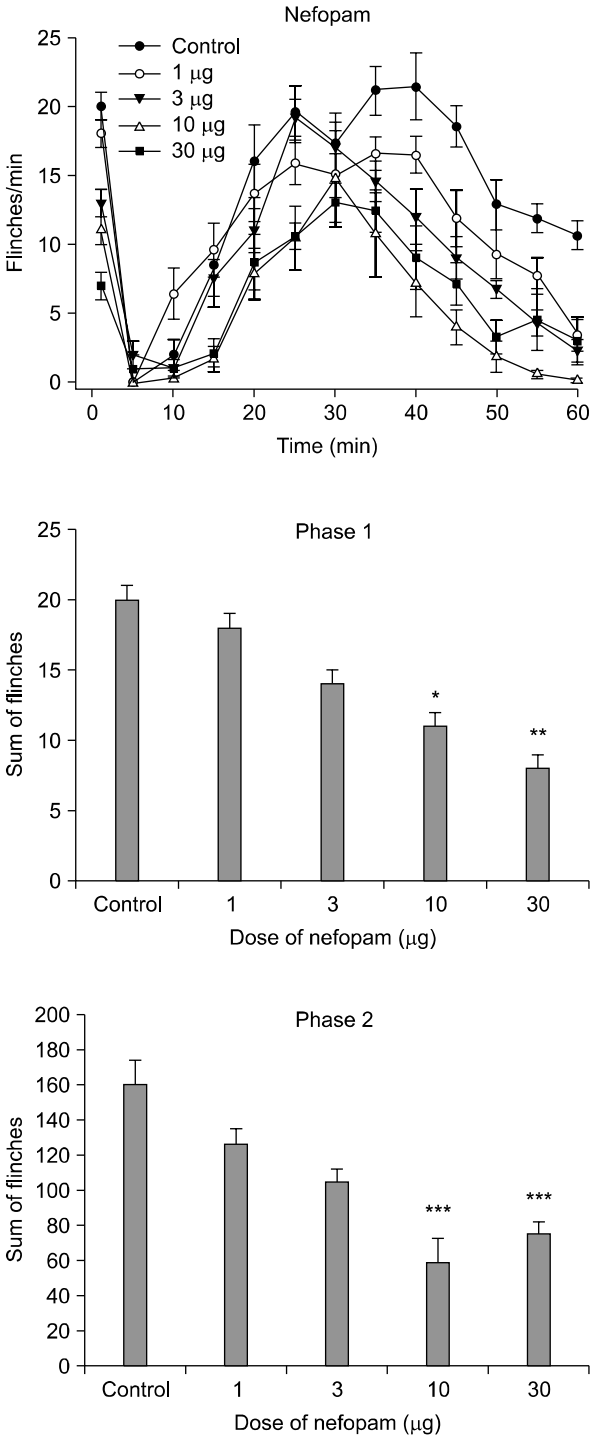

Fig. 2. Time course after formalin injection (top panel), and dose response curve (middle and bottom) are shown. Intrathecal nefopam significantly reduced the flinching responses in a dose dependent manner during both phases with peak effect at 30 μg. Formalin induced flinching responses are attenuated dose-dependently by intrathecal injection of morphine in a dose-dependent manner. Each line represents the mean ± SEM of 6–8 rats/group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. control.

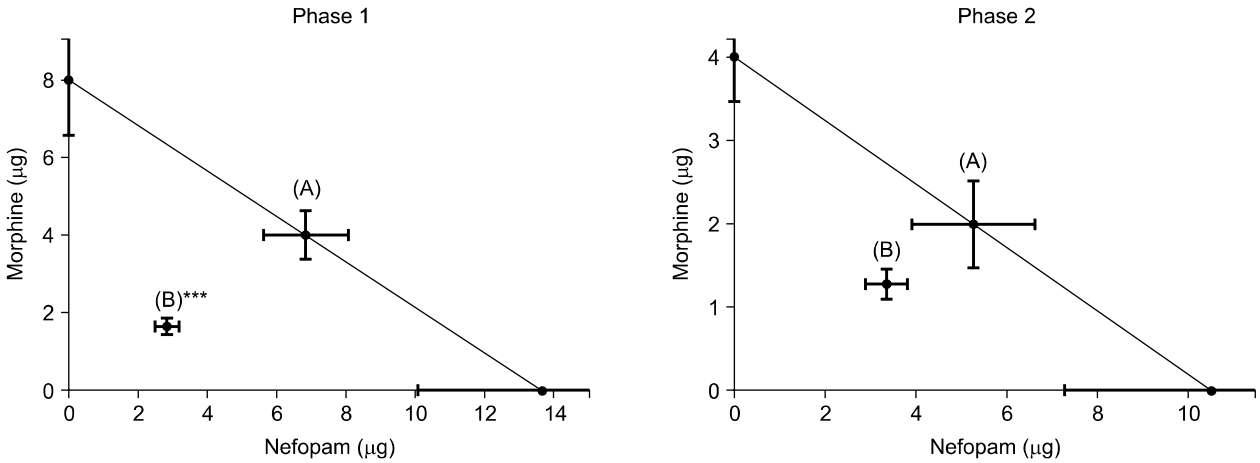

Fig. 3. Isobologram for the combined intrathecal administration of nefopam and nefopam during phase 1 (top panel) and phase 2 (bottom). ED_{50} with SEM of nefopam and morphine are plotted on x and y axes. The line connecting ED_{50} of nefopam and morphine on x and y axes is the theoretical additive line. The point (A) is the theoretical ED_{50} with SEM of drug combination, and point (B) represents the experimental ED_{50} with SEM. ***$P < 0.001$ vs. theoretical ED_{50}.

amine, has been suggested as a mechanism for nefopam-induced antinociception, despite the inconsistent results [2,20]. Moreover, most of the documented studies were performed on an *in vitro* basis, or on an *in vivo* pharmacological study using systemically or peripherally injected nefopam [21,22]. There have been a few reports on the analgesic effects of i.t. nefopam with equivocal results [3,13,14]. However, the spinal cord was also shown to be a site for analgesic action for nefopam [23], which is supported by the findings of this current study in which nefopam has a dose-dependent antinociceptive effect when administered intrathecally.

The effect of the combined administration of nefopam and morphine has been investigated in several animal pain models, as well as in postoperative pain or opioid-induced hyperalgesia [6-9,24,25]. While several studies have reported a morphine sparing effect of nefopam in a postoperative analgesia setting using the combination of a subanalgesic dose of nefopam or morphine, less or no significant effects of nefopam were observed [8,10-12]. Furthermore, a study using the isobolographic analysis revealed an infra-additive interaction in postoperative pain between intravenous nefopam and morphine [10]. The difference in pain modality originated from the different kinds of surgeries, which could at least partially explain the different results, since animal studies have shown that the descending modulations mediated by serotonin or noradrenalin, the main targets of nefopam, could be inhibitory or facilitatory depending on the pain stimuli [15].

As for animal studies, nefopam has shown to spare other analgesics including paracetamol, COX-2 inhibitor, or NSAIDs, or enhance the efficacy of these analgesics [25-28]. In contrast, the interaction between nefopam and morphine has not been examined in only a few studies, in which nefopam enhanced the analgesic effect of morphine in the pain elicited by chronic constriction injury and carrageenan injection [24,25]. These studies, however, used only a combination of the sub-analgesic dose for both drugs to examine the interaction. To the best of our knowledge, this study is the first to address the interactions of morphine and nefopam using the isobolographic analysis at the spinal level.

The different patterns of interaction in phase 1 and phase 2 are likely to reflect the different mechanisms of the nociceptive behavior [17,29]. The acute pain of phase 1 is derived from the direct stimulation of the peripheral nociceptor, and the facilitatd pain of phase 2 involves the increased excitability of the central neurons and the ongoing primary afferent input. Accordingly, the combination of nefopam and morphine could be regarded as being more useful for acute pain than chronic inflammatory pain. However, the additive interaction during phase 2 was different from the enhanced analgesic effect of morphine in the carrageenan-induced inflammatory pain. This discrepancy could be related to the previous findings, in which the descending serotonergic modulation is differentially in-

volved in inflammatory pain that is induced by formalin or carrageenan [30,31]. The analgesic interaction of nefopam with various drugs also seems to support the results of this current study, which displayed the different patterns of the interactions during phase 1 and 2. Furthermore, it was found to possess properties other than the inhibition of monoamine reuptake was suggested to mediate the antinociceptive effect of nefopam, which involves the glutamatergic system, transient receptor potential vanilloid subtype 1, and serotonin receptors [32-34]. These multiple mechanisms could contribute to the analgesic effect in many pain states, while interacting with other analgesics including morphine.

In conclusion, this study demonstrated that i.t. nefopam produces an antinociceptive effect in the behavior of formalin induced pain during both phase 1 and phase 2, while interacting with i.t. morphine synergistically during phase 1, and additively during phase 2. The results of this study support the usefulness of nefopam as a component of multimodal analgesia, and may facilitate further clinical studies for the optimal use of nefopam.

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